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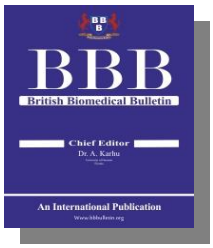

Original

In vitro Antifungal Potentials of Bioactive Compounds Heptadecane, 9- hexyl and Ethyl iso-allocholate isolated from *Lepidagathis cristata* Willd. (Acanthaceae) leaf.

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ABSTRACT

Objective: To identify bioactive compounds Heptadecane, 9- hexyl and Ethyl iso-allocholate from *Lepidagathis cristata* Willd.(*L. cristata*) leaf to assess antifungal potentials of the isolated compounds
Methods: Aqueous extracts of *L. cristata* leaves were used for this study. The major bioactive compounds isolated were tested for antifungal activities.
Results: The major bioactive compounds Heptadecane, 9- hexyl and Ethyl iso-allocholate were isolated from the leaves of *L. cristata* and these compounds were tested for antifungal potentials and found to be highly effective to plant pathogenic fungi *Colletotrichum fulcatum* NCBT 146, *Fusarium oxysporum* NCBT 156 and *Rhizoctonia solani* NCBT 196 as well as for the human pathogenic fungi *Curvularia lunata* MTCC 2030 and *Microsporum canis* MTCC 2820.
Conclusions: The results justify the antifungal potentials of both plant and human pathogenic fungi. The plant bioactive compounds will be helpful in herbal antifungal formulations.

Introduction

Plants are used as food, flavor, cosmetic, ornamental, fumigant, insect deterrent and medicine[1]. Our ancestors relied on the herbal products as therapeutic which can be traced back to at least 5,000 years[2]. Approximately 3000 plant species are known to have medicinal properties in India[3]. According to World Health Organization (WHO) about 80% of the world population depends on the natural products for their health due to minimal side effect and cost effective[4].

Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, medicinal plants and animals. Systemic

investigation of folk medicine may result in the discovery of novel effective compounds[1,5]. Therefore several medicinal plants have been evaluated for possible antimicrobial activity and get remedy from variety of antimicrobial origin[6].

Lepidagathis cristata Willd. (Acanthaceae) (*L. cristata*) is a medicinal herb and used as bitter tonic in fevers and used in pneumonia, flu, mouth infections[7], eczema, psoriasis and other skin infections[8]. The ash of whole herb is applied externally on chronic wounds of pet animals[9]. The roots of the herb are used in stomachic and dyspepsia, leaves are used for fevers and the inflorescence ash is used for itchy affections of skin and burns[10,11].

The plant is a stiff herb and the branches procumbently arise from a hard central rootstock. Leaves are alternate, elliptic, serrate and usually lineolate. Flowers are sessile, capitate, the heads terminal or axillary densely crowed at the base of the plant, fruits glucose capsule[12,13]. This medicinal herb has been exploited tremendously by common people in many ways for various curative purposes. It is necessary to evaluate the herb in a scientific base for its potential use of folk medicine for the treatment of infectious diseases[14].

Antibacterial studies[14,15], pharma-cognostical and phytochemical studies[16], analgestic and anti- inflammatory activities studies[17], hypoglycaemic activity in alloxan induced diabetic rats of *L. cristata* have been documented so far but antifungal activity of this herb seems to be lacking[18]. Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific bases[19]. Literature survey on *L. cristata* revealed that the therapeutic properties of this herb had not been established so far. Hence an attempt was made in the present study to investigate the feasibility of using *L.cristata* against various fungal isolates of both plant and human pathogens.

Materials and Methods

Collection and identification of plant material

Fresh plants of *L. cristata*, Willd. (Acanthaceae) were collected from Pachhaimalai Hills, Tiruchirappalli District, Tamil Nadu, India (Figure 1a). The taxonomic identities of the plant were confirmed by previously described[12]. The plant material was washed under running tap water; air dried in shade and then the leaf was homogenized to fine powder and stored in sterile air tight bottles for the experimental use.

Fungal cultures

The fungal cultures tested in this work *Colletotrichum fulcatum* NCBT 146 (*C. fulcatum*), *Fusarium oxysporum* NCBT 156 (*F. oxysporum*) and *Rhizoctonia solani* NCBT 196(*R. solani*) were maintained in immobilized condition in polyurethane foam in Microbiology Lab, Department of Biotechnology, National College, Tiruchirappalli, Tamil Nadu, India, where as *Curvularia lunata* MTCC 2030 (*C. lunata*) and *Microsporum canis* MTCC 2820 (*M. canis*) were obtained from Microbial Type Culture Collection and Gene Bank MTCC, Chandigarh, India.

Experimental procedure

Different weight of dry leaf powder (2 mg, 4mg, 6 mg and 12 mg) were mixed with different volume of Sabourand dextrose agar (SDA) medium (HI media M063) to form different concentrations (100 mg/L, 200 mg/L, 400 mg/L and 800 mg/L). The Control-1 contained only 20 ml of SDA medium and Control-2 contained 2 mg of bavistin fungicide added to 20 ml of SDA medium at 100 mg/L concentration. The leaf powder is mixed with the medium in

Petridish (9 cm) and inoculated with 0.5 ml spore suspension of fungi prepared from 10 days old culture. The experimental Petri dishes were incubated for 8 d at (28±2)°C temperature in dark. Three replicates were prepared and inoculated with fungal spores for each treatment.

Determination of the minimum inhibitory concentration (MIC)

MIC was determined by the liquid dilution method[20]. Dilution series were prepared with 0.25 to 15.00 mg/ml of Sabourand dextrose broth medium. To each tube 0.1 ml of standardized suspension of fungal spores (4× 10^6spores/ml) were added and incubated at (28±2)°C for 24 h. The lowest concentration which did not show any growth of the tested fungi after microscopic evaluation was determined as MIC.

Isolation of bioactive compound-Thin Layer Chromatography (TLC)

Glass plates (4 cm×12 cm) were used in which 30 g silica gel mixed with 60 ml distilled water and slurry was prepared and coated on the glass plate to 0.25 cm thickness dried for an hour at 110°C in an air oven[21].

Preparation of leaf extract for bioactive compound

The dry powdered leaf (500 mg) of *L. cristata* was mixed with 5.0 ml of chloroform and ground into a paste, dried at room temperature. About 1 ml of chloroform was added to the dried samples and spotted on the TLC plates. The TLC plates were kept in several eluent mixtures with different polarities to separate the bioactive chemical compounds. The eluent used were chloroform: n-hexane (8:2), chloroform: ethyl acetate (8:2), chloroform: acetone (8:2), n-hexane: acetone (9:1), and chloroform: acetone (9:1). Samples spotting on the TLC plate were done by using a micropipette in which the dot diameter was 0.5 mm. The chloroform: acetone (9:1) was the best eluent since it was able to separate the compounds contained in leaf extract[22].

Gas Chromatography and Mass Spectroscopy (GC-MS)

GC-MS analyses were performed using a GC Clarus 500 Perkin Elmer equipment, equipped with a flame ionization detector and injector MS transfer line temperature of 230°C, fused silica capillary column Elite-5 MS (5%diphenyl/95% dimethyl polysiloxane), 30.00×0.25 µl df, film thickness, carrier gas helium at a flow rate of 28cm/s was used. A volume of 1 ml of extract mixed with methanol (80%) at a split rate 10:1 was injected[23]. The compound identification was accomplished by comparing the GC relative retention and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices and by comparison to reference compounds.

Results and Discussion

The aqueous extract of dried powder of *L. cristata* leaf has shown varied antifungal properties against both plant pathogenic as well as human pathogenic fungi tested in this work (Table 1). The growth of both plant and human pathogenic fungal strains were totally inhibited at 800 mg/L. The total inhibition can be comparable to Control-2, a standard antifungal agent bavistin at 100 mg/L. However at 400 mg/L concentration 75% growth inhibition was exhibited by all fungal strains expect *F. oxysporum* NCBT 156 where total inhibition was noticed. At 200 mg/Lconcentration75% inhibition was exhibited by *F.oxysporum* NCBT 156, *M. canis* MTCC 2820, *R. solani* NCBT 194 and 50% inhibition for *C. fulcatum* NCBT 146, *C. lunata* MTCC 2030. About 50%

inhibition was observed in all fungal strains at 100 mg/L concentration (Figure 1b-1f)

The MIC values of the aqueous extract of leaf varied from 6.0 mg/ml to 13.0 mg/ml for the fungi tested. The MIC value of *R. solani*, *F. oxysporum*, *C. fulcatum*, *C. lunata* and *M. canis* were 6.0, 7.50, 10.0, 11.50 and 13.0 mg/ml respectively. Further investigation was performed to demonstrate the action of the extract on these fungi at different concentrations. The growth of these fungi correspondingly decreased with increasing concentration of the extract and the growth was completely inhibited at their MIC values. The reduction of growth was possibly due to the interference by active principles, i.e., bioactive compounds Heptadecane, 9- hexyl and Ethyl iso-allocholate (Table 2 and Figure 2). Therefore, the MIC determination is important in giving a guideline of the choice of an appropriate and effective concentration of antifungal therapeutic substance.

The results of earlier work with *L. cristata* reveal that the plant extract is significantly effective against Gram-positive bacteria[14,16,24,25]. Aqueous extract of leaves mixed with *Ocimum* juice in 10:1 ratio is used to cure fever[26] and leaf paste with coconut oil was applied externally on old wounds until cured[27]. The methanol, chloroform and ethyl acetate extracts of leaf has shown significant analgesic effect on albino mice[28,29]. Review of literature reveals information on the antifungal potential of *L. cristata* inflorescence extract[29]. In the present investigation the antifungal activity of its leaf has been demonstrated for the first time. *L. cristata* leaf extract was investigated for its potential bioactive compounds Heptadecane, 9- hexyl ($C_{23}H_{48}$) and Ethyl iso-allocholate ($C_{26}H_{44}O_5$) are effective plant extract and as antifungal agent for plant and human pathogenic fungi.

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Conflict of interest

We declare that we have no conflict of interest.

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Table 1. Antifungal potentials of bioactive compounds Heptadecane, 9- hexyl and Ethyl iso-allocholate isolated from leaf of *L. cristata*.

Fungus	Control		Concentration of the extract			
	1	2	100 mg/L	200 mg/L	400 mg/L	800 mg/L
C. fulcatum NCBT 146	+ + + +	-	+ +	++	+	-
C. lunata MTCC 2030	+ + + +	+	+ +	++	+	-
F.oxysporum NCBT 156	+ + + +	+	+ +	+	-	-
M. canis MTCC 2820	+ + + +	+	+ +	+	+	-
R. solani NCBT 194	+ + + +	-	+ +	+	+	-

Control-1: Medium without leaf extract; Control-2: Medium with Bavistin (100 mg/L). ++++: Normal growth; +++: 25% growth inhibition; ++: 50% growth inhibition; +: 75% growth inhibition; - : Total (100%) growth inhibition.

Table 2. Components identified in *L. cristata* leaf.

RT	Name of the compound	Molecular formula	MW	Peak area %
4.96	Heptadecane, 9- hexyl-	$C_{23}H_{48}$	324	24.41
5.55	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	9.45
7.63	Oleic acid, 3-(octadecyloxy) propyl ester	$C_{39}H_{76}O_3$	592	3.94
11.61	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	22.05
14.96	Rhodopin	$C_{40}H_{58}O$	554	8.66
24.67	Lycopene	$C_{40}H_{56}$	536	7.09
32.32	Stigmasterol	$C_{29}H_{48}O$	412	7.87
34.29	Tetrahydrospirilloxanthin	$C_{42}H_{64}O_2$	600	16.54

MW: Molecular weight. Parameters tested are not covered under the scope of NABL accreditation.

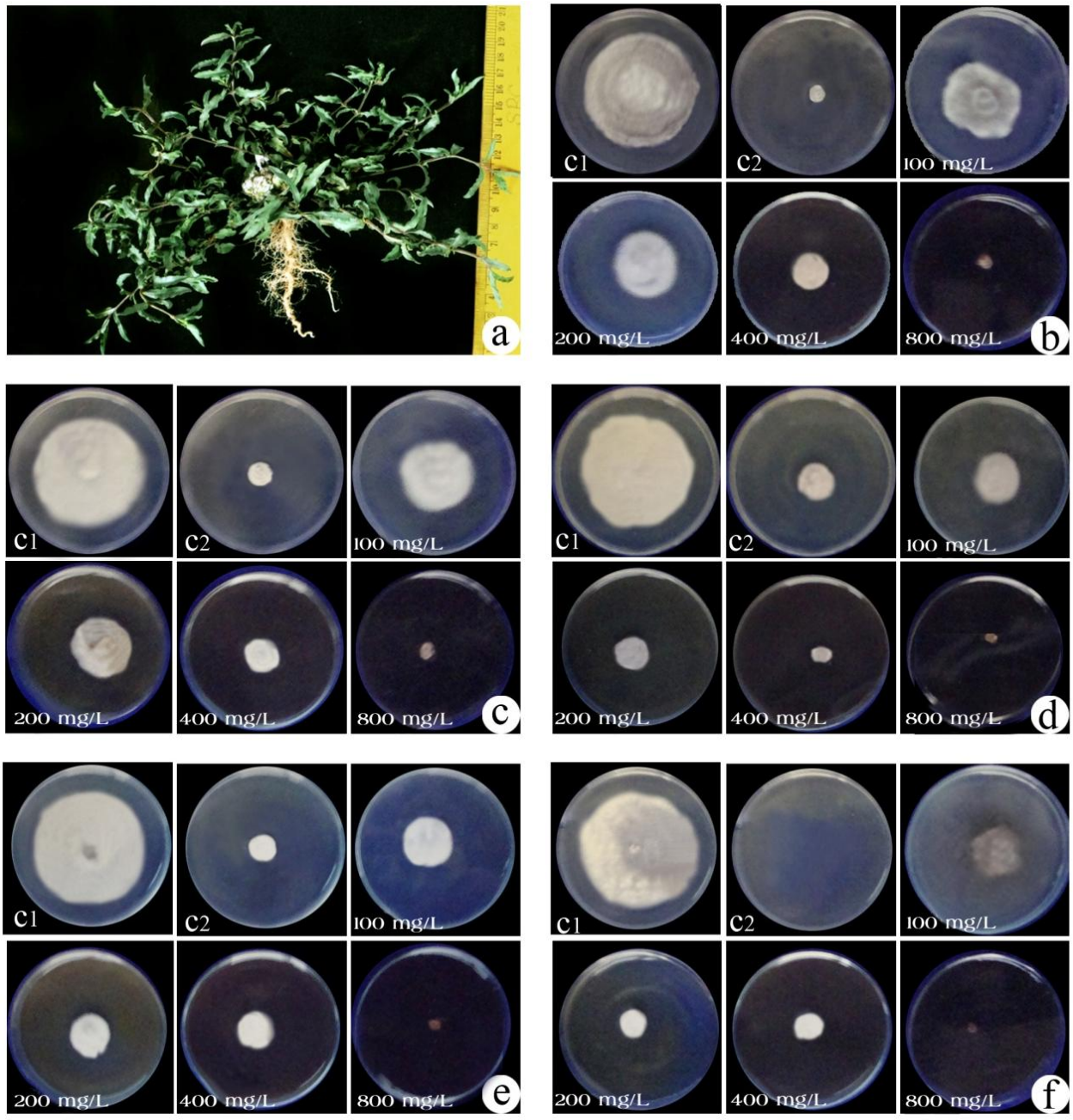

Fig. 1: Antifungal potentials of bioactive compounds Heptadecane, 9- hexyl- and Ethyl iso-allocholate isolated from *Lepidagathis cristata* Willd.(Acanthaceae) leaf.
a: *L. cristata* plant habit; **b:** *C. fulcatum* NCBT 146; **c:** *C. lunata* MTCC 2030; **d:** *F.oxysporum* NCBT 156; **e:** *M. canis* MTCC 2820; **f:** *R. solani* NCBT 194; **C1:** Control-1 (without leaf extract); **C2:** Control -2(Bavistin 100 mg/L). Concentrations of leaf extract ranged from 100mg/L to 800 mg/L.

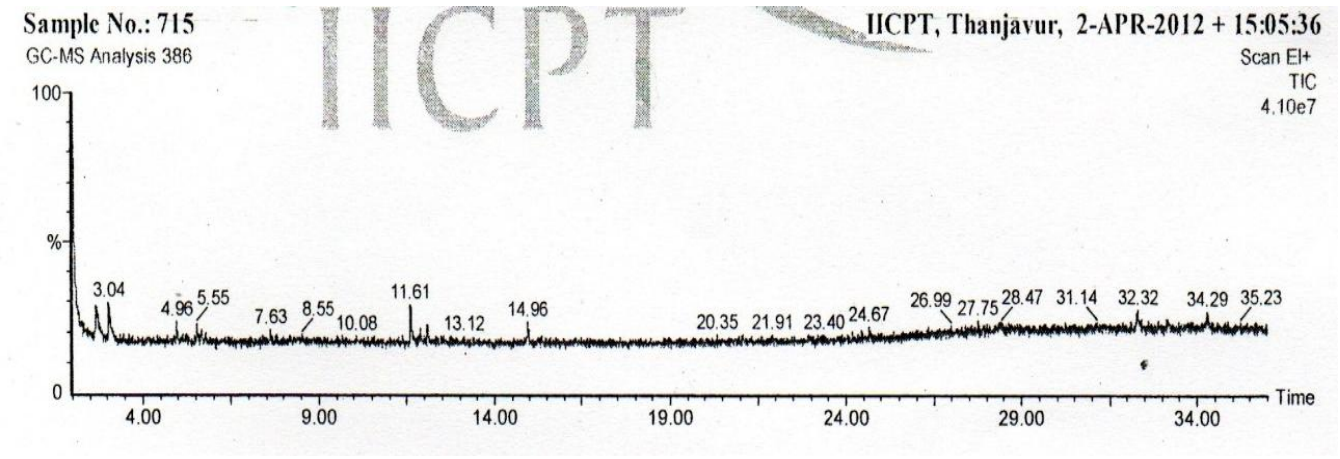

Fig. 2: GC-MS Chromatogram of *L. cristata* leaf.
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